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SEQUENCE LISTING

<110> SAHIN, ERINC
TARALP, ALPAY
SAYERS, SEHRA

<120> CIRCULAR RECOMBINANT PLASMID DNA CONSTRUCTS AND THEIR PROTEIN
PRODUCTS, METHODS OF PREPARATION AND IMMOBILISATION OF PROTEINS
ON SUPPORT

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<140> 10/550226
<141> 2005-09-20

<150> PCT/TR2003/000019
<151> 2003-03-20

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<170> PatentIn version 3.3

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 <223> pGFPuv plasmid coding for GFP from Aequorea victoria

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 <223> pGFPuv plasmid coding for GFP from Aequorea victoria

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Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
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ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa cgc act ata tct Phe Lys Ser Ala Met 90 Pro Glu Gly Tyr Val Gln 95 Glu Arg Thr Ile Ser 100	585
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cac aat gta tac atc acg gca gac aaa caa aag aat gga atc aaa gct His Asn Val Tyr Ile Thr 155 Ala Asp Lys Gln Lys Asn 160 Gly Ile Lys Ala	777
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35 40 45Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
145 150 155 160Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

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Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
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Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
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<221> CDS

<222> (1)..(876)

<223> pETM-GFP-Imm plasmid containing Hisx6 tag, flexible joint
as frame adapter, and A. victoria GFP gene

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Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
20 25 30

gta gaa aaa atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca 144
Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
35 40 45

att ctt gtt gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc 192
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
50 55 60

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Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65 70 75 80

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Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
85 90 95

act act ttc tct tat ggt gtt caa tgc ttt tcc cgt tat ccg gat cat 336
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
100 105 110

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115 120 125

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 20 25 30

Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
 35 40 45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
 50 55 60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
 65 70 75 80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
 85 90 95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
 Page 16

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
115 120 125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
130 135 140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145 150 155 160

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
165 170 175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
180 185 190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
195 200 205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
210 215 220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225 230 235 240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
245 250 255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
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result of plasmid modification

<220>

<221> misc_feature
 <222> (1)..(17)
 <223> Frame adapter used for prevention of frameshift mutation as a
 result of plasmid modification

<400> 8
 catgggaggc acgttac

17

<210> 9
 <211> 5
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Peptide design based on size and flexibility to act as a linker
 between the tag and GFP protein segments

<220>
 <221> MISC_FEATURE
 <223> Peptide design based on size and flexibility to act as a linker
 between the tag and GFP protein segments

<400> 9

Met Gly Gly Thr Val
 1 5

<210> 10
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 <212> PRT
 <213> Artificial sequence

<220>
 <223> Peptide design based on charge and shape to bind the
 expressed protein to a suitably interactive surface

<220>
 <221> MISC_FEATURE
 <223> Peptide design based on charge and shape to bind the
 expressed protein to a suitably interactive surface

<400> 10

His His His His His His
 1 5

<210> 11
 <211> 238
 <212> PRT
 <213> Aequorea victoria

<220>
 <221> MISC_FEATURE
 <223> Green fluorescent peptide coded by pGFPuv plasmid, permitting
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easy visualisation and quantification based on fluorescence
properties

<400> 11

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 12
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 <212> PRT
 <213> Artificial sequence

<220>
 <223> Completed peptide based on Hisx6 tag, short physically flexible linker and green fluorescent protein coded by pETM-GFP-Imm to impart fluorescence properties, allowing easy immobilisation with retention of bioactivity, visualisation and quantification

<220>
 <221> MISC_FEATURE
 <223> Completed peptide based on Hisx6 tag, short physically flexible linker and green fluorescent protein coded by pETM-GFP-Imm to impart fluorescence properties, allowing easy immobilisation with retention of bioactivity, visualisation and quantification

<400> 12

Met Lys His His His His His His Pro Met Ser Asp Tyr Asp Ile Pro
 1 5 10 15

Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
 20 25 30

Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
 35 40 45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
 50 55 60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
 65 70 75 80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
 85 90 95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
 100 105 110

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
 115 120 125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
 130 135 140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
 145 150 155 160

015936-2.ST25.txt

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
165 170 175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
180 185 190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
195 200 205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
210 215 220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225 230 235 240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
245 250 255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
260 265 270

Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Pro Leu Arg
275 280 285

Ser Gly Cys
290

<210> 13
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<212> DNA
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<220>
<223> Frame adapter

<400> 13

gtacgccatg ggaggcacgg taccttggtg

29